# Protective effect of microbial phytase on vital organs of cadmium chloride-treated mice: A histopathological study.

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## Abstract

The present study was conducted to investigate the role of dietary microbial phytase supplementation in ameliorating the toxic effects of gradual doses of cadmium chloride on vital organs. For this purpose, 80 albino male mice were randomly assigned to 8 equal groups (intact control and 7 treated groups). Intact control (IC) was supplemented with feed and water without any addition, Ph group was supplemented with phytase in diet (600 PU/kg), Cd1 group was orally administered with $CdCL_2$ (0.03 mg/L), Cd1-Ph group was orally administered with $CdCL_2$ (0.03 mg/L) and supplemented with phytase in diet (600 PU/kg), Cd2 group was orally administered with $CdCL_2$ (5.0 mg/L), Cd2-Ph group was orally administered with $CdCL_2$ (5.0 mg/L) and supplemented with phytase in diet (600 PU/kg), Cd3 group was orally administered with $CdCL_2$ (5.0 mg/L), Cd2-Ph group was orally administered with $CdCL_2$ (5.0 mg/kg) and supplemented with phytase in diet (600 PU/kg). The experiment extended for 30 days. After the last day of experiment, mice were anesthetized, dissected, and livers, kidneys spleens, lungs, bone marrows and ileums were obtained for histopathological examination. Blood vessels congestion and hemorrhage have been appeared in $CdCl_2$ treated groups which increased with gradual doses of $CdCl_2$ in each of livers, Kidneys, and lungs. Kidneys showed amyloid deposition in glomeruli and renal tubules. Renal tubular epithelial cells suffered from necrosis and there was necrosis in many nuclei. The spleen showed hyperplasia and distortion of normal micro-architecture. There was degenerative cells in payers patches in ileum. Bone marrow revealed decrement in blood forming cell which replaced by fat cells. Dieting microbial phytase supplementation exhibited a protective role against these toxic effects.

## Introduction

Cadmium is one of the most dangerous occupational and environmental toxics. It is found in drinking water, atmospheric air, and even in food (Kowalczyk *et al.,* 2003). Products of vegetable origin are the main carrier of cadmium compound in food (Kies, 2001) having been absorbed from the alimentary tract. Cadmium forms durable combinations with protein thionein, forming metallothionins which play an important role in further metabolism of this metal. Cadmium causes poisoning in various tissues of humans and animals (Stohs *et al.,* 2001).

Kidneys (Mainly renal cortex) and liver are considered to be the most susceptible organs in the case of exposure to cadmium (Ryan *et al.,* 2000). Cadmium half-life is about 10 years, so the symptoms of cadmium intoxication may occur several years after the exposure (Kowalczyk *et al.,* 2003). In recent years in scientific investigations attention has been drawn to the "health-promoting" activity of microbial phytase supplementation diet. Microbial phytase has the specific role of catalysts for break down the links between phosphorus and phytate ring in phytic acid and therefore liberating the phytin phosphorus (Kerovou, 2000). Also microbial phytase was eamined by researchers to reduce cadmium toxic effects (Rimbach *et al.,* 1995). Bilal and Ercag (2003) observed that microbial phytase reduced cadmium accumulation in liver and kidney by up 60%. In view of the possible involvement of microbial phytase in ameliorating the toxic damage of cadmium chloride in male mice, in which detail mechanisms are not completely understood, and the potential for the application of microbial phytase as antitoxic agent, this study aims to investigate the effect of microbial phytase supplementation on histopathological changes of vital organs in mice treated with gradual doses of cadmium chloride.

## Materials and methods

**Chemicals:** Pure cadmium chloride 0.5 hydrate $CdCl_2.0.5\ H_2O$. with a molecular weight of 2/0.33 gm was used. (RIEDE-DE-HAENAG, Germany). Microbial phyaste Biofeed® phytase Wovozyme® Denemark.

## Animals and Experimental Designs

Mature Swiss white Balb/C with mean mass 25±2 g were supplied by Iraqi center for embryologic research and sterility, ministry of health, Iraq. Male mice were allowed one week to acclimate to the animal house environment before beginning of experiment. Animals were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 22±2 °C., the light-dark cycle was on a 12:12 hr with light on at 06:00 a.m. and off at 06:00 p.m. throughout the experimental period. A total of 80 mice were assigned into 8 equal groups and treated for 30 days as follow:

1) **IC group:** intact control, was supplemented with feed and water without any addition.

2) **Ph group:** was supplemented with phytase in diet (600 PU/kg).

3) **Cd1 group:** was orally administered with $CdCL_2$ (0.03 mg/L).

4) **Cd1-Ph group:** Cd1-Ph group was orally administered with $CdCL_2$ (0.03 mg/L) and supplemented with phytase in diet (600 PU/kg).

5) **Cd2 group:** Cd2 group was orally administered with $CdCL_2$ (5.0 mg/L).

6) **Cd2-Ph group:** Cd2-Ph group was orally administered with $CdCL_2$ (5.0 mg/L) and supplemented with phytase in diet (600 PU/kg).

7) **Cd3 group:** Cd3 group was orally administered with $CdCL_2$ (5.0 mg/L).

8) **Cd3-Ph group:** Cd2-Ph group was orally administered with $CdCL_2$ (5.0 mg/kg) and supplemented with phytase in diet (600 PU/kg).

To have 600 PU/kg diet phytase in the diets, 120 mg Biofeed ® phytase Nodozyme ® (*Aspergillus niger*, containing 5000 pu/g phytase activity) was used. PU/ phytase unite:- the amount pf phytase that liberate/mm inorganic phosphate from 1.5 Mm of sodium phosphate at a constant time (1 minute) with pH 5.5 and temperature 37ºC (Engelen *et al.,* 1994).

Twenty four hours after the last day of experiment, mice were anesthetized with ether inhalation, dissected, and livers, Kidneys, spleens, Lungs, ileum, epiphysis of femurs were immediately removed and fixed with neutral buffered formalin 10%. Samples were dehydrated with in 50% to 100% alcohol. And xylene was used for clearing samples. Femoral bone was decalcified using 105 formic acid. Tissue were embedded in paraffin, sectioned with microtome; 5 mm thick, stained with H & E and examined with light microscope (Luna, 1986).

**Results**

**Kidneys**: There are no notable changes in the renal cortex including glomeruli and tubular epithelium in the IC and Ph groups (fig. 1a). In Cd1, Cd2, and Cd3 groups showed moderate blood vessels congestions and hemorrhage in the interstates (fig. 1b). The damaged renal cortex were observed mainly in Cd2 and Cd3 groups which represented by amyliod deposition in the tubular epithelium and glomeruli, severe vascular congestion and tubular degeneration (fig.1c and 1d). All these changes appeared to be less detected in the groups supplemented with phytase plus cadmium chloride (fig.1e).

**Liver:** The hepatocytes showed normal histology in the specimens obtained from IC and Ph groups (fig.2a). The hepatic sinusoids were hemorrhaged and showed vascular congestion in Cd1 group (fig.2b). Cd2 group showed hemorrhage in the portal tract and sinusoids and hepatocytes had condensed nuclear chromatin pattern consistent with pyknosis (fig.2c), whereas in Cd3

group, the defects were observed more frequently in sever hemorrhage between sinusoids, over hepatocellular necrosis, hemosiderin pigmentation and increased numbers of activated kupffer cells (fig.2d). Whereas mice fed on the microbial phytase with the diet are partially reversed these changes (fig. 2e).

**Spleen:** The splenic white and red pulp showed normal histology in the specimens obtained from IC, Ph, Cd1, Cd1-Ph, Cd2, and Cd2-Ph groups (fig.3a), whereas the lesions appeared in the highest dose of cadmium chloride (Cd3 group) inludes hyperplasia and normal splenic architecture was moderately distorted (fig.3b). Whereas dietary phytase supplementation reversed these effects to the control levels (fig.3c).

**Lungs:** The alveoli and the alveolar wall appeared normal in IC and Ph groups (fig.4a). Vascular congestion and moderate hemorrhage appeared in the alveoli of Cd1 group (fig.4b). The lesion was progressively increased in Cd2 group. (fig.4c). Severe vascular congestion, severe hemorrhage, distortion of normal alveolar architecture, alveolar degeneration, fibrosis of lungs, and swollen of alveolar walls were observed in Cd3 group (fig.4d) compared with that supplemented with microbial phytase (fig.4e).

**Bone marrow:** The pathological lesion in the bone marrow and reduction in the number of blood forming cells were observed in Cd2 group (fig.5b) compared with that of IC and Cd1 groups (fig.5a). This lesions appeared more frequently in Cd3 group including replacement of blood forming cells with fatty cells whereas some of bone marrow cavity appeared clear and empty spaces (fig.5c) in comparison with that of Cd3-Ph group (fig.5d).

**Ileum:** Ileum mucosa and lymphatic nodules (Payer′s patches) appeared normal in IC, Ph, Cd1, Cd1-Ph, Cd2, Cd2-Ph, and Cd3-Ph groups (fig.6a), whereas Cd3 group showed hyperplasia in lymphatic nodules (payer′s patches) and distortation of the normal architecture (fig.6b).

**Discussion**

The present study reported that cadmium chloride induced several histopathological consequences in vital organs and tissues of the body, including vascular congestion, hemorrhage, hepatocytes necrosis, inflammatory cell infiltration and tubular degeneration. Histopathological changes were gradually increased in its severity with the increased doses of cadmium chloride, which was still can be ameliorated, particularly cadmium chloride was used in subtoxic doses. The observed vascular congestion of liver, kidney, lungs, which represent the hyperemia (Anderson, 1980) and hemorrhage, especially in the portal tract of the liver were due to exudation of inflammatory cells (MacSween & Whaley, 1992). Amyliodosis may result from

loss of cells and damage of tissue (Tizand, 1990). Highest cadmium dose produced significant renal pathology including tubular cell degeneration and vacillation and tubular cell atrophy (Liu *et al.,* 1998). This results agreed with many researcher reports (Liu *et al.,* 2000, Jarup *et al.,* 2000, Jarup, 2002). It has been known for long time that cadmium mainly accumulates in liver and kidneys because these organs contain most of metallothionein binding toxic metals (Coudhury *et al.,* 2001; Liu *et al.,* 1998). In this study, hepatocellular necrosis, hepatocytes had a condensed nuclear chromatin pattern with pyknosis. Such lesions some times progress obviously in mice treated with highest dose of cadmium, also increased numbers of activated kupffer cells characterized by vaculated nuclei (Uyanik *et al.,* 2001). Kamiyama *et al.,* (1995) reported that kupffer cells are stimulated to produce cytokines such as TNF-α and 1L-6 after cadmium administration, and these cytokines are responsible for certain manifestation of liver damage. We also reported splenic histopathology included hyperplasia of the white pulp, such lesions also observed in the Payer′ patches in the in the ileum. This reflects immunological response to cadmium intoxication. The spleen is hemopoidic tissue in mice (Sty & Conway, 1985), so, any deviation in splenic-micro architecture will reflex positively on haemopoiesis. Bone marrow, considered a major site of haemopoiesis, also showed a marked reduction in blood-forming cells. This may be result in decreased haemopieosis (Machalinska *et al.,* 2002).

On the other hand, in this investigation, phytase supplementation succeeded in relieving the previously mentioned toxic effects of cadmium and ameliorated these changes to the normal. Although, little is known about the effect of dietary microbial phytase supplementation on the cadmium accumulation (Bilal and Ercag, 2003), their multidirectional activates account for the application of phytase against cadmium toxicity. This might be explained by the release of phytate bound antagonists of cadmium, minerals and trace elements especially calcium, zinc and iron, which are known to reduce the bioavailability and counter act the absorption of cadmium in different animal species (Kemme *et al.,* 1998; Jongbloed *et al.,* 2000; Zacharias *et al.,* 2001).

It can be concluded from presented results that cadmium chloride at different doses induced pathological lesions in vital organs of mice. Our results also showed that dietary supplementation of 120 mg of phytase per kg of diet compensates these effects and expressed protective role against toxic influences of cadmium.

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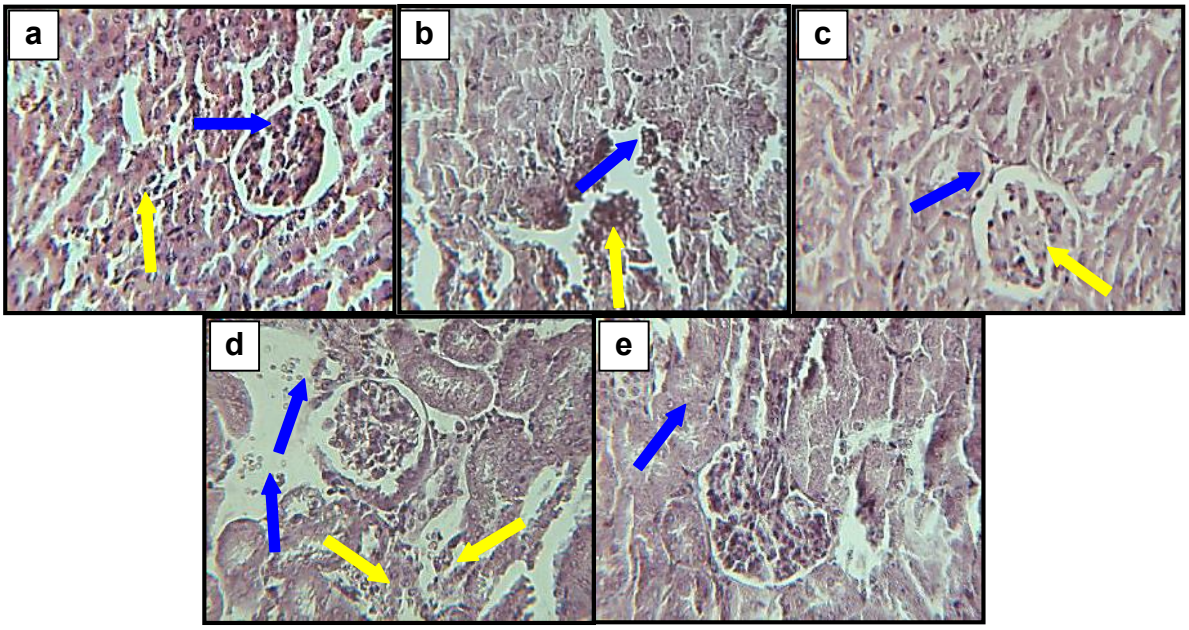


**Figure (1)**: sections obtained from cortex of kidneys; **a**: IC group shows glomerulus (blue arrow) and tubular cells (yellow arrow); **b**: Cd1 group shows congestion of blood vessels (blue arrow) and hemorrhage in the interstitium (yellow arrow); **c**: Cd2 group shows amyloid deposition in glomerulus (yellow arrow) and in tubule (blue arrow); **d**: Cd3 group shows Severe hemorrhage in the interstitium (blue arrow) and tubular cell degeneration (yellow arrow); **e**: Cd3-Ph group appeared to be less defect and shows mild deposition of amyloid (blue arrow). H & E x 400.

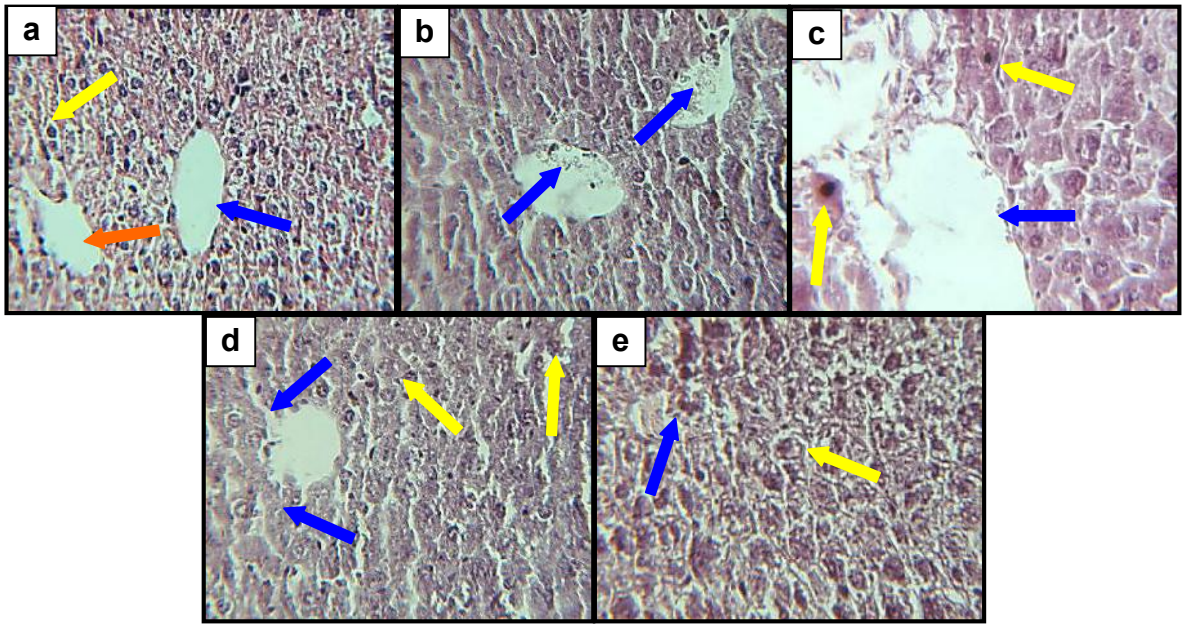


**Figure (2)**: sections obtained from livers; **a**: IC group shows central vein (blue arrow), hepatocytes (yellow arrow) and sinusoid (orange arrow); **b**: Cd1 group shows congestion of blood vessels and hemorrhage in the sinusoids (blue arrow); **c**: Cd2 group shows hemorrhage in the portal space (blue arrow) and pyknosis in some hepatocytes (yellow arrows); **d**: Cd3 group shows apoptosis of some hepatocytes (blue arrow) and increase distribution of Kupffer cells (yellow arrow), **e**: Cd3-Ph group appears less affected hepatocytes (yellow arrow) with mild hemorrhage between sinusoids (blue arrow). H & E x 400.

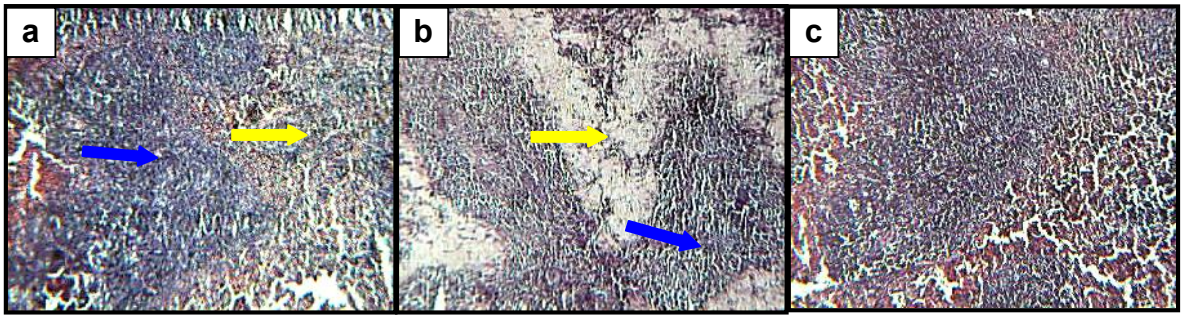


**Figure (3)**: sections obtained from spleens; **a**: IC group normal white pulp (blue arrow) and red pulp (yellow arrow); **b**: Cd3 group shows hyperplasia (blue arrow) and loss of architecture and depletion on lymphocytes and supportive tissues (yellow arrow); **c**: Cd3-Ph group appears less affected and lock-like normal appearance as that of control. H & E x 400.

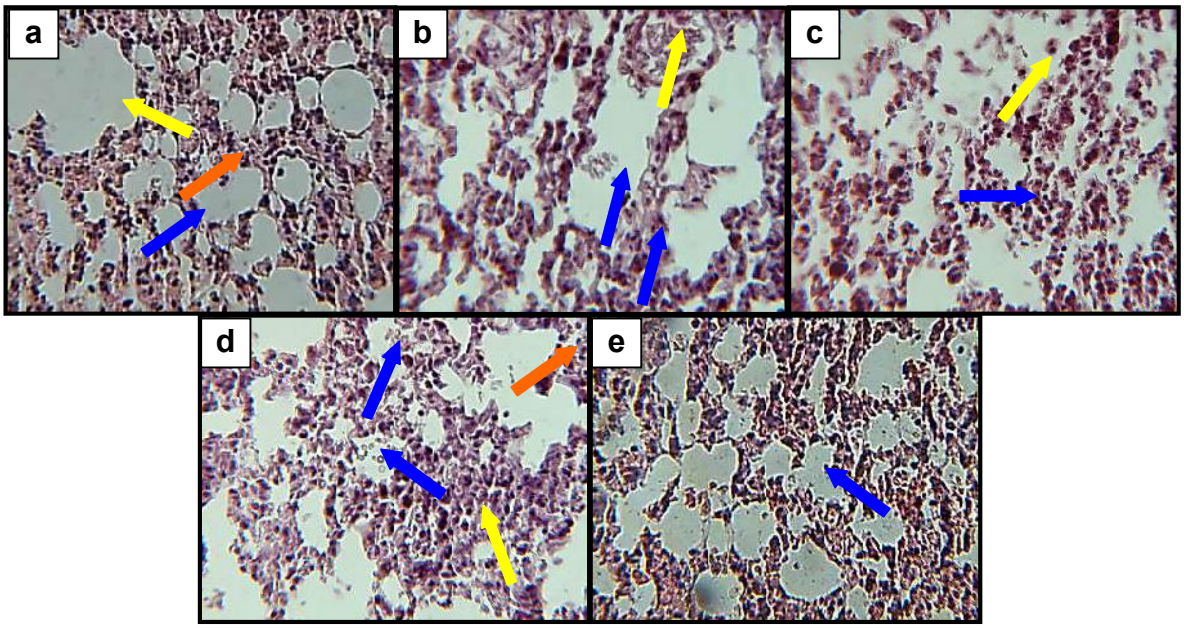


**Figure (4)**: sections obtained from lungs; **a**: IC group shows pulmonary alveoli (blue arrow), alveolar sac (yellow arrow) and lining of alveolar wall (orange arrow); **b**: Cd1 group shows congestion of blood vessels (blue arrow) and hemorrhage (yellow arrow); **c**: Cd2 group shows sever congestion between alveoli (yellow arrow) and destruction of alveolar walls (blue arrows); **d**: Cd3 group shows sever congestion between alveoli (yellow arrow), destruction and thickening of some alveolar walls (blue arrows), and presence of fibrosis (orange arrow), **e**: Cd3-Ph group appears less affected with the presence of mild congestion between alveoli (blue arrow). H & E x 400.

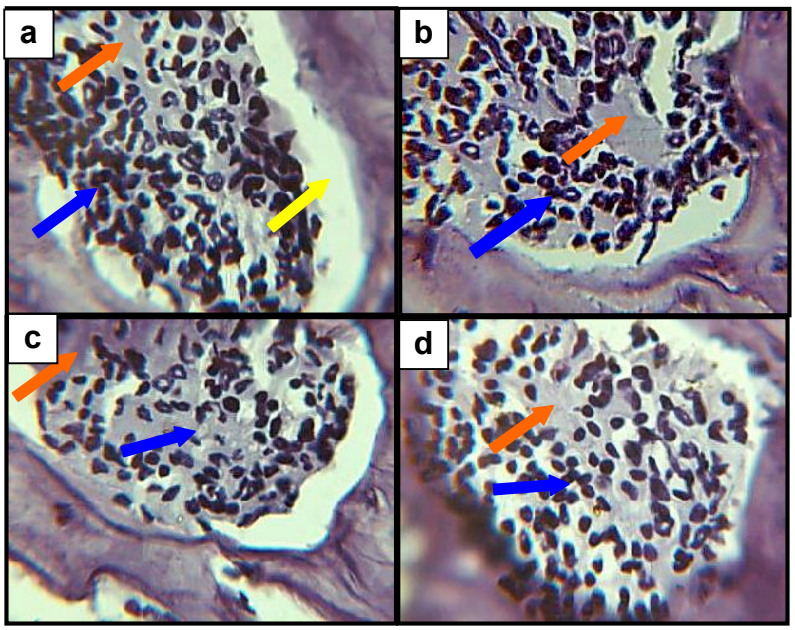


**Figure (5)**: sections obtained from red bone marrows; **a**: IC group shows hemopoietic cells (blue arrow), bone marrow caviety (yellow arrow) and fat cells alveolar wall (orange arrow); **b**: Cd2 group shows constriction of hemopoietic cells number (blue arrow) and increase in fat cell number (orange arrow); **c**: Cd3 group shows sever depletion of hemopoietic cells (blue arrow) and increase in fat deposition (orange arrow); **d**: Cd3-Ph group appears to be reversed nearly to the normal with increased number of hemopoietic cells (blue arrow) and some fat deposition (orange arrow). H & E x 400.

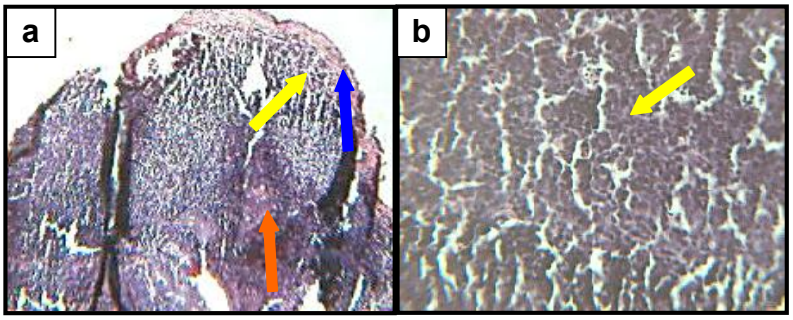


**Figure (6)**: sections obtained from ileums; **a**: IC group shows mucosa (blue arrow), submucosa (yellow arrow) and Payer's patches (orange arrow) which was nearly similar in all other groups except that of Cd3 group (**b)** which shows hyperplasia inside Payer's patches (yellow arrow) and deformation of some lymphocytes. H & E x 400.